

correlated with transgene copy number, thereby allowing high levels of expression at high transgene copy numbers.

Introduction of a transgene into the fertilized egg of an animal (e.g., a mammal) is accomplished by any number 5 of standard techniques in transgenic technology. See, e.g., Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1986. Most commonly, the transgene is introduced into the embryo by way of microinjection.

10 Once the transgene is introduced into the egg, the egg is incubated for a short period of time and is then transferred into a pseudopregnant animal of the same species from which the egg was obtained (Hogan et al., *supra*). In the case of mammals, typically 125 eggs are injected per 15 experiment, approximately two-thirds of which will survive the procedure. Twenty viable eggs are transferred into pseudopregnant mammal, four to ten of which will develop into live pups. Typically, 10-30% of the pups (in the case of mice) carry the transgene.

20 To identify the transgenic animals of the invention, progeny are examined for the presence of the transgene using standard procedures such as Southern blot hybridization or PCR. Expression of the transgene can also be assessed using Northern blots, Western blots, and immunological assays.

25 Without further elaboration, it is believed that one skilled in the art can, based on the above disclosure and the description below, utilize the present invention to its fullest extent. The following example is to be construed as merely illustrative of how one skilled in the art can 30 practice the invention and are not limitative of the remainder of the disclosure in any way. Any publications cited in this disclosure are hereby incorporated by reference.

Production of Transgenic Mice

Transgenic mice were produced by microinjection of DNA fragments into the pronuclei of fertilized mouse eggs as described in Brinster et al., Cell 27:223-231, 1981 and 5 Costantini et al., Nature 294:92-94, 1981. Plasmids pHS40- $\zeta$ 597-GH and pHS40(r-mt 1)- $\zeta$ 597-GH are described in Zhang et al., Mol Cell Biol 13:2298-2308, 1993. Digestion of these plasmids with EcoRI, NdeI, and ScaI yielded 3.12 kb DNA fragments containing the HS-40 enhancer, the  $\zeta$ -globin promoter, and the growth hormone (GH) open reading frame. 10 The 3.12 kb DNA fragments were eluted from soft agarose gels, purified, and used for microinjection.

Transgenic founders were identified and their transgene copy number determined by Southern blot analysis 15 of tail DNA. The founders were then bred with nontransgenic C57/B6 mice to establish lines. The morning on which the copulatory plug was observed was designated 0.5 day postcoital. For analysis of fetal (14.5 days postcoital) and embryonic (9.5 days postcoital) mice, transgenic males 20 were mated to nontransgenic C57/B6 females. Transgenic pups were identified by PCR analysis of fetal mice tails or of embryo DNA. For each identification, duplicate PCR reactions were carried out using one 5' primer from the  $\zeta$ -globin promoter region, and two different 3' primers from 25 the GH region (see below).

A total of 9 founders with wild type HS40- $\zeta$ GH (wt) and 10 founders with the mutant HS40- $\zeta$ GH (mt) have been obtained. The copy numbers of integrated fragments in wthS-40-containing mice vary from 1 to more than 100, as 30 shown in Table 1.

Table 1

Mutant HS-40 Transgene			Wild Type HS-40 Transgene			
Founder line	Copy number	hGH, ng/ml	Founder line	Copy number	hGH, ng/ml	
5	1A*	1	470	1A*	1	36
	1B*	1	530	1B*	1	20
	1C*	1	1,060	2	2	14
	2	2	650	3	3	22
	3	3	1,260	5	5	5
	8*	8	2,990	10*	10	13
	10*	10	3,360	13*	13	187
	13*	13	4,650	100A	>100	1,400
	15*	15	5,560	100B	>100	30
	19*	19	6,490			

15 In Table 1, the founders for which lines have been  
 established are indicated by an asterisk. Mice with the  
 wtHS-40 transgene were assayed at the age of 5 months except  
 founder 1B, which was evaluated at 9 months old. Mice with  
 the mtHS-40 transgene were assayed at the age of 4 months  
 except founder 15, which was evaluated at 2 months old.

20 The  $\zeta$ -globin promoter activities in the founder mice  
 were first measured with a blood GH assay as described in  
 Zhang et al., supra. The levels of human GH in the blood  
 were quantitated with the Allegro hGH radioimmunoassay kit  
 25 from Nichols Institute. When the concentration of GH in the  
 blood exceeded 50 ng/ml, the samples were first diluted with  
 horse serum in order to perform the assay in a linear range.

It was known that the amount of secreted enzyme  
 molecules are good representations of the quantities of